Research Article
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# Effects of whey added to drinking water of lambs on serum mineral concentrations

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Received on: 12/03/2022 Accepted on: 19/06/2022 Published on: 22/06/2022

## ABSTRACT

**Aim:** The study was aimed to reveal positive contributions related to consumption of whey which has high biological value and many important benefits, as drinking water in sheep, on serum mineral levels of animals.

**Method and materials:** Twenty-four healthy, 3-month-old, weaned male Merino lambs were randomly divided into 2 equal groups. Tap water was given *ad libitum* to the control group and drinking water prepared by adding whey to the whey group *ad libitum*. The total trial period was 45 days. The first 15 days were evaluated as the adaptation period of the lambs to the whey. On the 0$^{th}$, 15$^{th}$ and 30$^{th}$ days of the next 30 days, blood was drawn from the vena jugularis of all lambs. Serum calcium, magnesium, copper, zinc, and selenium levels, excluding serum phosphorus, were not significantly different between lambs consuming whey or tap water and between 3 blood draw days within each group.

**Results:** Although serum phosphorus levels were not significantly different between the 2 groups and between the days of bloodletting in the lambs consuming tap water, they increased significantly on the 15$^{th}$ day of the trial and decreased again on the 30$^{th}$ day in the whey-consuming group. The reduction at day 30 was not significantly different from day 0 and day 15.

**Conclusion:** Whey added to the drinking water and given to the lambs did not significantly affect the serum mineral levels examined, except for phosphorus. The significant increase in serum phosphorus concentration on the 15$^{th}$ day of the study in the whey group was thought to be due to the phosphorus contained in the whey, and the regression seen on the 30$^{th}$ day was evaluated as the body's adaptation. Although serum mineral levels did not change, it can be recommended to feed lambs with whey, as it is predicted that whey may contribute to the storage of minerals in tissues.

**Keywords:** Lamb, serum mineral, whey

**Cite This Article as**: Altiner A, Bilal T, Eseceli H and Danyer E (2022). Effects of whey added to drinking water of lambs on serum mineral concentrations. J. Vet. Res. Adv., 04(01): 27-33.

## Introduction

Whey is the byproduct of casein precipitation, which is an important step in cheese production (Mota et al., 2006). Whey proteins make up about 20% of total bovine milk proteins and have gained popularity as functional foods (Zhao et al., 2005). The main components of bovine whey proteins are β-lactoglobulin (55-60%) and α-lactalbumin (15-20%) (Prieto et al., 2007). β-lactoglobulin is the main component of whey protein. β-lactoglobulin has the best amino acid ratio and high branched chain amino acid content. β-lactoglobulin can bind soluble nutrients such as vitamin A and vitamin E.



It is an excellent source of α-lactalbumin, essential amino acids and branched-chain amino acids, and is the only whey protein component with the ability to bind metals and calcium (Ronghui, 2015). Minor components include bovine serum albumin, lactoferrin, immunoglobulins, glycomacro - peptides, phosphopoproteins, bioactive factors and enzymes (Prieto et al., 2007). Whey proteins contain all the essential amino acids the body needs, as well as high levels of lysine and arginine. Whey proteins are also a good source of sulfur-containing amino acids such as cysteine and methionine (Ronghui, 2015). Whey is also a good source of calcium, phosphorus, sulfur and water-soluble vitamins (Schingoethe, 1976). About 40% of the original milk's calcium and 43% of its phosphorus are found in the whey from Cheddar cheese (Price, 1944).

Whey has become a valuable food ingredient due to its excellent nutritional value and functional properties (Mota et al., 2006). Whey has the ability to act as an antioxidant, antihypertensive, antitumor, hypolipidemic, antiviral, antibacterial and chelating agent (Marshall, 2004) . Whey proteins are not only easy to digest but also have a high metabolic efficiency, so the proteins have high potency bioavailability (Ronghui, 2015). Various functional and biological activities of these proteins have been reported, including opioid inhibition, angiotensin 1-converting enzyme inhibition, antihypertensive activity, immunomodulatory activity, bacteriostatic activity, and downregulation of fatty acid synthesis in the liver (Costa et al., 2007).

The biological components of whey, including lactoferrin, β-lactoglobulin, α-lactalbumin, glycomacropeptide, and immunoglobulins, display a number of immune-enhancing properties (Marshall, 2004). It has been found that α-lactalbumin can regulate breast lactose synthesis and anticancer function. Whey has low lactoferrin content, but has high biological activity. Lactoferrin is antibacterial, inhibits the formation of free radicals, regulates body iron transfer, promotes cell growth and enhances immunity, promotes the proliferation of bifidobacteria, and is an antioxidant (Ronghui, 2015). Whey is a good source of energy, especially due to its high lactose content (Schingoethe, 1976).

The primary mechanism thought to exert the effects of whey is the intracellular conversion of the amino acid cysteine to glutathione, a potent intracellular antioxidant (Marshall, 2004). Glutathione is essential for the antioxidant defense system, immune system cells, and skeletal muscle cells (Ronghui, 2015). Whey proteins may reduce stress and depression by lowering cortisol and increasing brain serotonin, improve liver function in hepatitis, help with chronic fatigue, improve athletic performance, and lower blood pressure. Whey proteins are a complete protein containing 8 essential amino acids and are more widely used than other proteins, especially in individuals who do weight training (Aragon and Schoenfeld, 2013). Whey proteins contain an optimal balance of amino acids for muscle growth, specifically glutamine or glutamic acid and taurine. There is evidence that whey proteins and vitamin D can positively affect muscle mass or function (Siddiqui et al., 2008).

Epidemiological and animal studies show an inverse relationship between dairy product intake and the accumulation or loss of body fat mass (Teegarden, 2005). In humans, intake of calcium and dairy products at 2-year intervals has been associated with reduced body fat mass accumulation (Lin et al., 2000). Evidence in rodent models has shown that high calcium or dairy diets prevent fat mass accumulation (Papakonstantinou et al., 2003).

Significant amounts of whey and whey products can be used in animal feed. Whey can be used in animals in a variety of ways, such as liquid whey, condensed whey, dried whey, or dry whey products. Fermented, ammoniacal condensed whey is an acceptable liquid protein supplement for ruminants. Liquid whey can serve as a satisfying feed for growing calves and heifers. Rumen butyrate is usually increased when diets contain whey or whey products (Schingoethe, 1976). In this study, it was aimed to reveal the positive contributions that the consumption of whey, which has high biological value and many important benefits, as drinking water in sheep, to the serum mineral levels of animals.

## Materials and Methods

*Selection of Animals*

Twenty-four healthy male Merino lambs, 3 months old, weaned, housed in a private farm, were randomly divided into 2 equal groups.

*Feeding*

All lambs were fed with concentrate and roughage twice a day (at 08:00 and 17:00). Lambs were given lamb grower feed as concentrate and wheat straw as roughage. The control group was given *ad libitum* tap water as drinking water. Drinking water prepared by adding 6.56 g of whey powder purchased from Astosan (Gonen, Balikesir) to 100 L of tap water was given to the whey group *ad libitum*. The chemical analysis results of the lamb grower feed components and the lamb grower feed, wheat straw and whey powder used in the study were recorded (Tables 1 and 2), respectively.

*Experimental design*

The total trial period was 45 days. The first 15 days were evaluated as the adaptation period of the lambs to the whey. On the 0$^{th}$, 15$^{th}$ and 30$^{th}$ days of the next 30 days, blood was collected from the vena jugularis of all lambs with 8 ml vacuum and gel tubes (Vakutest® Kima, Italy). Then, the blood was brought to the laboratory by cold chain and centrifuged at 2000 g for 10 minutes (Hettich® Rotofix 32A, Kirchlengern, Germany). Blood serums were transferred to 2 ml eppendorf serum storage tubes and stored in a deep freezer at -80 °C until analysis. When assays were to be made, the sera were slowly thawed at 4 °C.

Table 1. Components of lamb grower feed used in the study (Eseceli et al., 2021).

| Components | g/kg | Components | g/kg |
|---|---|---|---|
| Wheat bran | 251.0 | Rice bran | 35.5 |
| Corn grain | 170.0 | Limestone | 31.0 |
| Cornflour | 127.3 | Ammonium chloride | 6.0 |
| Barley | 100.0 | Molasses | 5.0 |
| Sunflower seed meal | 96.4 | Provin | 5.0 |
| Boncalite | 80.0 | Salt | 3.0 |
| Flaxseed meal | 50.0 | Vitamin + mineral premix* | 1.5 |
| Distillers dried grains with solubles | 38.3 | | |

*Vitamin + mineral premix contains 150 mg $ZnSO_4.7H_2O$, 80 mg $MnSO_4.H_2O$, 200 mg MgO, 5 mg $CuSO_4.7H_2O$, 1 mg $KIO_3$, 5000 IU vitamin A, 1000 IU vitamin D, and 20 IU vitamin E.

Table 2. Chemical composition of lamb grower feed, wheat straw and whey powder used in the study (Eseceli et al., 2021).

| Chemical composition | Lamb grower feed | Wheat straw | Whey powder |
|---|---|---|---|
| Dry matter (fed basis), g/kg | 875.8 | 927.0 | 974.1 |
| Crude protein, g/kg | 225.2 | 48.0 | 174.3 |
| Crude ash, g/kg | 95.1 | 76.1 | 85.3 |
| Ether extractives, g/kg | 75.9 | 16.0 | 19.0 |
| Crude fiber, g/kg | 83.3 | 323.6 | - |
| Neutral detergent fiber, g/kg | 227.3 | 730.5 | - |
| Acid detergent fiber, g/kg | 105.1 | 494.4 | - |
| Acid detergent lignin, g/kg | 40.4 | 88.0 | - |
| Non-fiber carbohydrate, g/kg | 376.3 | 129.4 | 721.4 |
| Metabolizable energy, kcal/kg | 3052.1 | 2439.7 | 3057.2 |

*Tests:* Serum calcium, phosphorus and magnesium analyzes were performed using a fully automated dry chemistry analyzer (Fujifilm® DRI-Chem NX500i, Fuji Medical System Co., Ltd., Tokyo, Japan). Serum copper, zinc and selenium analyzes were performed with an inductively coupled plasma mass spectrometer device (Agilent 7700x ICP-MS, Agilent Technologies, Santa Clara, CA, Unites States) after the serum samples were burned in the microwave (Berghof Microwave, Berghof Instruments, Eningen, Germany).

*Statistical analysis:* Statistical analysis was performed via IBM® SPSS® Statistics for Windows (V. 21, Armonk, NY, USA). Data are expressed as mean ± standard deviation and the differences between the days were tested with the analysis of variance (one-way ANOVA) followed by the Tukey post-hoc test. The differences between the groups were tested with independent sample t test. $p<0.05$ was considered as statistically significant difference.

## Results and Discussion

Mean serum mineral levels and standard deviations and statistical comparisons between 2 groups or within each group between days in lambs that consume whey or tap water as drinking water (Table 3).

As can be seen from the table, serum calcium, magnesium, copper, zinc and selenium levels, excluding serum phosphorus, were not significantly different between lambs consuming whey or tap water and between 3 blood draw days within each group. Although serum phosphorus levels were not significantly different between the 2 groups and between the days of bloodletting in the lambs consuming tap water, they increased significantly on the 15$^{th}$ day of the trial and decreased again on the 30$^{th}$ day in the whey-consuming group. The reduction at day 30 was not significantly different from day 0 and day 15.

In nature, all members of the animal and plant kingdom need minerals for their survival and efficient performance. The minerals were present in cells and tissues of animals in certain concentrations for normal growth, health and productivity of animals (Ranjith and Pandey, 2015). Adequate protein intake is essential for bone health (Rizzoli and Bonjour, 2004). There was a positive relationship between the mineral mass of bone and dietary protein intake (Rapuri et al., 2003). A low protein diet is associated with increased bone resorption and decreased bone formation (Ammann et al., 2001). Dietary proteins affect both the production of IGF-I and its effect on bone anabolism (Rizzoli and Bonjour, 2004). Whey protein is a good source of highly bioavailable calcium (Ronghui, 2015).

Table 3. Serum mineral levels in lambs consuming whey or tap water as drinking water

| Minerals | Groups | Day 0 | Day 15 | Day 30 |
|---|---|---|---|---|
| Calcium, mg/dl | Control<br>Whey | 12.11 ± 1.19<br>11.93 ± 0.88 | 11.66 ±0.70<br>12.00 ± 0.47 | 11.80 ± 0.33<br>11.74 ± 0.54 |
| Phosphorus, mg/dl | Control<br>Whey | 9.45 ± 1.94<br>8.23 ± 2.47[a] | 10.01 ± 1.21<br>11.16 ± 1.20[b] | 10.51 ± 0.67<br>9.38 ± 3.28[ab] |
| Magnesium, mg/dl | Control<br>Whey | 2.90 ± 0.44<br>2.93 ± 0.27 | 2.96 ± 0.20<br>2.95 ± 0.27 | 3.04 ± 0.17<br>3.12 ± 0.31 |
| Copper, ppm | Control<br>Whey | 1338.78 ± 657.77<br>938.18 ± 302.78 | 848.31 ± 304.84<br>1027.60 ± 263.07 | 891.90 ± 670.10<br>1110.41 ± 491.91 |
| Zinc, ppm | Control<br>Whey | 870.86 ± 166.55<br>639.45 ± 251.94 | 606.15 ± 247.19<br>946.31 ± 228.26 | 683.55 ± 277.48<br>1025.71 ± 645.16 |
| Selenium, ppm | Control<br>Whey | 135.58 ± 31.89<br>111.80 ± 35.85 | 107.58 ± 24.78<br>159.93 ± 33.70 | 135.23 ± 55.18<br>196.60 ± 94.43 |

Mean ± standard deviation, n = 12, [a, b]: There is a significant difference between the values indicated by different letters in the row ($p<0.05$).

Vandenplas et al. (1993) fed the infants with whey-predominant formula or whey hydrolysate formula from birth until they are 3 months old, and they found that plasma calcium concentrations in the 3rd month were not significantly different between the 2 groups (10.5 vs 10.6 mg/dl, respectively). In the study of Seppo et al. (2005), infants with cow milk allergy were fed soy formula or hydrolyzed whey formula from 7.5 months to 2 years of age. Blood was drawn from the infants at 7.5 months, 1st and 2nd years of age. Serum calcium levels were not significantly different between the 2 groups and between the days of blood draw within each group. Similarly, Kawase et al. (2000) gave men fermented milk supplemented with whey protein concentrate twice a day for 8 weeks. Serum calcium concentrations were not significantly different between the milk-drinking and non-milk-drinking groups at weeks 4 and 8.

Zhao et al. (2005) fed infant rats with calcium-enriched whey protein concentrate in their study. Whey protein concentrate increased intestinal calcium absorption (18-36%) in acute feeding (5 hours), but did not affect intestinal calcium absorption due to adaptation in chronic feeding (8 weeks). When calcium was not added, whey protein concentrate did not affect bone or calcium metabolism. Kopeć et al. (2014) also fed 5-week-old rats with whole wheat bread made with sourdough with or without whey protein, and they did not find the serum calcium concentration significantly different between the 2 groups (2.70 vs 2.78 µmol/L, respectively). Similar to the findings of Seppo et al. (2005), Kawase et al. (2000), Zhao et al. (2005), and Kopeć et al. (2014), serum calcium concentrations were not significantly different between groups and between the within-group days in the current study. Although increased dietary protein intake may improve intestinal absorption of calcium (Kerstetter et al., 2003), this was not reflected in serum calcium levels. The reason for this may be the adaptation of the body to chronic nutrition with whey (Zhao et al., 2005).

Whey proteins have a phosphorus/protein ratio of <1 mg/g compared to egg albumin (<5 mg/g), whole egg (<15 mg/g) and animal proteins (7-29 mg/g) (Kalantar-Zadeh et al., 2010). Vandenplas et al. (1993), fed infants with whey-predominant formula or whey hydrolysate formula from birth to 3 months of age and found that 3-month plasma phosphorus concentrations were not significantly different between the 2 groups. Kawase et al. (2000) gave men fermented milk containing whey protein concentrate twice daily for 8 weeks. Serum phosphorus concentrations at weeks 4 and 8 were not significantly different between milk-drinking and non-milk-drinking groups. Kopeć et al. (2014) fed 5-week-old rats with whole wheat bread made of sourdough with whey protein or without whey protein and they did not find the serum phosphorus concentration significantly different between the 2 groups (2.61 vs 2.79 µmol/L, respectively). In contrast, Hall et al. (1984) fed 3-8 weeks old infants with low birth weight with calcium- and phosphorus-supplemented soy isolate formula or whey-predominant premature formula for 8 weeks. While the researchers did not find the serum phosphorus concentrations significantly different between the 2 groups at the 3rd and 5th weeks, they found the concentrations to be significantly higher in the whey-predominant premature formula group at the 7th and 8th weeks than in the other group.

In the present study, serum phosphorus concentrations were not significantly different between the 2 groups at days 0, 15, or 30. At the same time, the levels were not significantly different between days in the control group. In the present study, no significant difference was found between the 2 groups in terms of serum phosphorus concentrations, as similar to the findings of Kawase et al. (2000), Kopeć et al. (2014), and Hall et al. (1984). In the studies of Kawase et al. (2000), serum phosphorus concentrations were found to be significantly higher in the group that drank fermented milk added the whey protein concentrate in the 4th week compared to the 0th week. In the 8th week, the levels regressed to the levels in the 0th week. Similarly, in the current study, the levels were found to be significantly higher on the 15th day than on the 0th day in the whey group. On the 30th day, the levels regressed to the initial levels. The significant increase detected on the 15th day may be due to the phosphorus content of the whey, and the decrease on the 30th day may be due to the adaptation of the body (Zhao et al., 2005).

Kopeć et al. (2014) fed 5-week-old rats with whole wheat bread made with sourdough with or without whey protein, and they could not find a significant difference in serum magnesium concentration between the 2 groups (1.32 vs 1.36 µmol/L, respectively). Kawase et al. (2000) gave men fermented milk containing whey protein concentrate twice a day for 8 weeks. Serum magnesium concentrations at weeks 4 and 8 were not significantly different between the milk-drinking and non-milk-drinking groups. In addition, no significant differences were found in serum magnesium concentration between 0, 4 and 8 weeks within each group. In the current study, the absence of significant differences in serum magnesium levels between whey-drinking and non-whey-drinking groups and between blood collection days within each group is similar to the findings of Kopeć et al. (2014) and Kawase et al. (2000).

Transition metals such as iron and copper are vital for multiple oxidation states and steps of electron transfer reactions. These metals participate in cellular processes such as oxygen transport, photosynthesis, nitrogen fixation, and respiration in most organisms (Uriu-Adams and Keen, 2005). Seppo et al. (2005) administered soy formula or hydrolyzed whey formula to infants with cow milk allergy from 7.5 months to 2 years of age. Blood was drawn from the infants at 7.5 months, 1st and 2nd years of age. Serum copper levels were not significantly different between the 2 groups and between the days of blood draw within each group. In the current study, the absence of significant differences in serum copper levels between whey-drinking and non-whey-drinking groups and between blood collection days within each group is similar to the findings of Seppo et al. (2005).

Zinc has a pronounced effect on bone metabolism by inhibiting bone resorption and stimulating bone formation (Yamaguchi and Uchiyama, 2005). Seppo et al. (2005) administered soy formula or hydrolyzed whey formula to infants with cow milk allergy from 7.5 months to 2 years of age. Blood was drawn from the infants at 7.5 months, 1st and 2nd years of age. Serum zinc levels were not significantly different between the 2 groups and between the days of blood draw in each group. Researchers have reported that serum zinc concentration cannot be considered as a very good indicator of nutritional status as it is affected by many factors such as acute stress, infections and growth rate. Rodondi et al. (2009) stated that since most of the zinc stores in the body are intracellular, plasma zinc measurements may not be a reflection of body stores. The absence of significant differences in serum zinc levels between whey-drinking and non-whey-drinking groups and between blood collection days within each group was similar to the findings of Seppo et al. (2005).

## Conclusion

As a result, whey added to drinking water and given to lambs did not significantly affect serum mineral levels, except phosphorus. The significant increase in serum phosphorus concentration on the 15th day of the study in the whey group was thought to be due to the phosphorus contained in the whey, and the regression seen on the 30th day was evaluated as the body's adaptation. Although serum mineral levels did not change, it can be recommended to feed lambs with whey, as it is predicted that whey may contribute to the storage of minerals in tissues.

## Reference

Ammann P, Rodriguez L, Aubert ML, Bonjour JP and Rizzoli R (2001). Growth hormone (GH) decreases bone mineral mass and bone strength in adult female rats fed a low protein diet. J. Bone Miner. Res., 16(Suppl. 1): S294.

Aragon AA and Schoenfeld BJ (2013). Nutrient timing revisited: is there a post-exercise anabolic window? J. Int. Soc. Sports Nutr., 10(5): 1-11.

Costa EL, Rocha-Gontijo JA and Netto FM (2007). Effect of heat and enzymatic treatment on the antihypertensive activity of whey protein hydrolysates. Int. Dairy J., 17: 632-640.

Eseceli H, Esen S, Keten M, Altiner A and Bilal T (2021). Effect of whey protein-enriched water on performance and in vivo carcass measurements in fattening Merino lambs. Alinteri J. Agr. Sci., 36(1): 61-65.

Hall RT, Callenbach JC, Sheehan MB, Hall FK, Thibeault DW, Kurth CG and Bowen SK (1984). Comparison of calcium- and phosphorus-supplemented soy isolate formula with whey- predominant premature formula in very low birth weight infants. J. Pediatr. Gastroenterol. Nutr., 3(4): 571-576.

Kalantar-Zadeh K, Gutekunst L, Mehrotra R, Kovesdy CP, Bross R, Shinaberger CS, Noori N, Hirschberg R, Benner D, Nissenson AR and Kopple JD (2010). Understanding sources of dietary phosphorus in the treatment of patients with chronic kidney disease. Clin. J. Am. Soc. Nephrol., 5(3): 519-530.

Kawase M, Hashimoto H, Hosoda M, Morita H and Hosono A (2000). Effect of administration of fermented milk containing whey protein concentrate to rats and healthy men on serum lipids and blood pressure. J. Dairy Sci., 83(2): 255-263.

Kerstetter JE, O'Brien KO and Insogna KL (2003). Dietary protein, calcium metabolism, and skeletal homeostasis revisited. Am. J. Clin. Nutr., 78(3 Suppl): 584S-592S.

Kopeć A , Borczak B , Pysz M, Sikora E, Sikora M, Curic D and Novotni D (2014). An addition of sourdough and whey proteins affects the nutritional quality of wholemeal wheat bread. Acta Sci. Pol. Technol. Aliment., 13(1): 43-54.

Lin YC, Lyle RM, McCabe LD, McCabe GP, Weaver CM and Teegarden D (2000). Dairy calcium is related to changes in body composition during a two-year exercise intervention in young women. J. Am. Coll. Nutr., 19(6): 754-760.

Marshall K (2004). Therapeutic applications of whey protein. Altern. Med. Rev., 9(2): 136-156.

Mota MVT, Ferreira IMPLVO, Oliveira MBP, Rocha C, Teixeira JA, Torres D and Goncalves MP (2006). Trypsin hydrolysis of whey protein concentrates: characterization using multivariate data analysis. Food Chem., 94: 278-286.

Papakonstantinou E, Flatt WP, Huth PJ and Harris RBS (2003). High dietary calcium reduces body fat content, digestibility of fat, and serum vitamin D in rats. Obes. Res., 11(3): 387-394.

Prieto CA, Guadix A, González-Tello P and Guadix EM (2007). A cyclic batch membrane reactor for the hydrolysis of whey protein. J. Food Eng., 78: 257-265.

Price WV, G Bohstedt G and Rupel JW (1944). Whey for livestock. Univ. WI Circular, 340.

Ranjith D and Pandey JK (2015). Mineral profiles in blood and milk of sheep. IJSR, 4(10): 821-826.

Rapuri PB, Gallagher JC and Haynatzka V (2003). Protein intake: effects on bone mineral density and the rate of bone loss in elderly women. Am. J. Clin. Nutr., 77(6): 1517-1525.

Rizzoli R and Bonjour JP (2004). Dietary protein and bone health. J. Bone Miner. Res., 19(4): 527-531.

Rodondi A, Ammann P, Ghilardi-Beuret S and Rizzoli R (2009). Zinc increases the effects of essential amino acids-whey protein supplements in frail elderly. J. Nutr. Health Aging, 13(6): 491-497.

Ronghui S (2015). The reasearch on the anti-fatigue effect of whey protein powder in basketball training, Open Biomed. Eng. J., 9: 330-334.

Schingoethe DJ (1976). Whey utilization in animal feeding: A summary and evaluation. J. Dairy Sci., 59(3): 556-570.

Seppo L, Korpela R, Lönnerdal B, Metsäniitty L, Juntunen-Backman K, Klemola T, Paganus A and Vanto T (2005). A follow-up study of nutrient intake, nutritional status, and growth in infants with cow milk allergy fed either a soy formula or an extensively hydrolyzed whey formula. Am. J. Clin. Nutr., 82(1): 140-145.

Siddiqui SMK, Chang E, Li J, Burlage C, Zou M, Buhman KK, Koser S, Donkin SS and Teegarden D (2008). Dietary intervention with vitamin D, calcium, and whey protein reduced fat mass and increased lean mass in rats. Nutr. Res., 28(11): 783-790.

Teegarden D (2005). The influence of dairy product consumption on body composition. J. Nutr., 135(12): 2749-2752.

Uriu-Adams JY and Keen CL (2005). Copper, oxidative stress, and human health. Mol. Aspects Med. 26(4-5): 268-298.

Vandenplas Y, Hauser B, Blecker U, Suys B, Peeters S, Keymolen K and Loeb H (1993). The nutritional value of a whey hydrolysate formula compared with a whey-predominant formula in healthy infants. J. Pediatr. Gastroenterol. Nutr., 17(1): 92-96.

Yamaguchi M and Uchiyama S (2005). Regucalcin stimulates osteoclast-like cell formation in mouse marrow cultures. J. Cell Biochem., 94(4): 794-803.

Zhao Y, Martin BR, Wastney ME, Schollum L and Weaver CM (2005). Acute versus chronic effects of whey proteins on calcium absorption in growing rats. Exp. Biol. Med. (Maywood), 230(8): 536-542.

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